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ORIGINAL ARTICLE

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Update on the role of antiseptics in the management of chronic wounds with critical colonisation and/or biofilm

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Abstract

Biofilms play a major role in delaying chronic wounds from healing. A wound infiltrated with biofilm, or "critically colonised" wound, may become clinically infected if the number of microbes exceeds a critical level. Chronic wound biofilms represent a significant treatment challenge by demonstrating recalcitrance towards antimicrobial agents. However, a "window of opportunity" may exist after wound debridement when biofilms are more susceptible to topical antiseptics. Here, we discuss the role of antiseptics in the management of chronic wounds and biofilm, focusing on povidone-iodine (PVP-I) in comparison with two commonly used antiseptics: polyhexanide (PHMB) and silver. This article is based on the literature reviewed during a focus group meeting on antiseptics in wound care and biofilm management, and on a PubMed search conducted in March 2020. Compared with PHMB and silver, PVP-I has a broader spectrum of antimicrobial activity, potent antibiofilm efficacy, no acquired bacterial resistance or cross-resistance, low cytotoxicity, good tolerability, and an ability to promote wound healing. PVP-I represents a viable therapeutic option in wound care and biofilm management, with the potential to treat biofilm-infiltrated, critically colonised wounds. We propose a practical algorithm to guide the management of chronic, non-healing wounds due to critical colonisation or biofilm, using PVP-I.

KEYWORDS

biguanides, biofilms, povidone-iodine, silver, wound healing

1 | INTRODUCTION

Wound healing is a complicated and tightly regulated process that is essential to restore the normal barrier function of the skin, thereby preventing further damage or infection.[1,2] The normal wound healing process involves sequential but overlapping phases, including inflammation, proliferation, and remodelling.[1] These phases are mediated by a range of cell types including fibroblasts, keratinocytes, endothelial cells, and macrophages, the activity of which is carefully coordinated by a range of growth factors, cytokines, and chemokines.[3] However, when a wound fails to progress through the normal successive phases of wound healing in an orderly and timely manner, a chronic wound may result.[1,4] Chronic wounds, comprising vascular leg ulcers (eg, venous and arterial

ulcers), diabetic foot ulcers, and pressure ulcers, represent a significant burden to the individual and to the healthcare system.[5,6] There are many factors that may be responsible for the delay and/or failure of a chronic wound to heal, including the patient's age, the nutritional status (eg, obesity) of the patient, how oxygenated the wound is, and the presence of either an underlying chronic disease (eg, diabetes) or an immunocompromised condition (eg, cancer).[7,8] Certain therapies may also hinder wound healing, including chemotherapeutic agents, radiation therapy and long-term use of corticosteroids, and non-steroidal anti-inflammatory drugs.[9,10]

Perhaps the most important factor that can affect the healing of a chronic wound, and subsequently increase the likelihood the wound may become infected, is the presence of a biofilm.[11-14] Biofilms are generally polymicrobial communities, attached either to each other or to a surface that become encased within an extracellular polymeric substance (EPS).[11,15,16] Most biofilms located within chronic wounds are composed of 10% to 20% microorganisms and 80% to 90% EPS.[11] Biofilms cause chronic wounds to become locked in an inflammatory state,[17,18] and they are thought to be the root cause of approximately 80% of all infections in humans,[11] and most medical device-related infections.[19] A recent systematic review and meta-analysis has proposed that the prevalence of biofilms in chronic wounds is 78.2%, although a more conservative estimate suggests that the figure may be 60%.[13,20] Biofilms in chronic wounds are difficult to visualise macroscopically, and whether located on the surface of a wound or deeper in the wound bed, the identification and diagnosis of biofilms in chronic wounds can be challenging.[21] Although tissue biopsies are regarded as one of the most reliable methods to detect biofilms in wounds, the procedure can be invasive, painful, and expensive, and usually requires a specifically trained practitioner to be performed.[22-24] Furthermore, the distribution of biofilms within chronic wounds is not thought to be uniform,[25] so a single biopsy sample taken from just one small area of a wound may not be enough to confirm the presence of biofilm within the wound as a whole. An algorithm to help identify (and treat) suspected biofilms has been developed, which is intended to guide the clinical management of chronic wounds.[11] In addition, recent consensus guidelines have been proposed to help clinicians recognise the signs and symptoms of biofilms in chronic, non-healing wounds, thereby optimising patient care.[21] Nevertheless, there are currently no "gold standard" diagnostic tests to enable clinicians to confirm the presence of biofilms in chronic wounds.[11,18,21,26]

The "wound infection continuum" conceptualises the relationship between bacteria, wound, and host,[12] and a key phase of this continuum is "critical colonisation", which marks the proliferation of microbes within the wound and the development of biofilm.[8] Agreement is yet to be reached on how best to define the term critical colonisation, but Cutting (2003) suggested it refers to a wound that has become compromised by microbes, resulting in delayed healing without causing the classic signs and symptoms traditionally associated with clinical infection.[27] A critically colonised—or more generally biofilm infiltrated—wound has the potential to deteriorate to clinical infection once microbes reach a critical level (10^5 colony-forming units per gram tissue); should the numbers of bacteria increase above this level, the likelihood of infection increases as the host's immune system may no longer be able to control the proliferating microbes.[8,28] In contrast, an infected wound marks the presence of multiplying microbes, which have already overwhelmed the host's immune system, leading to the traditional clinical signs of inflammation, including redness, swelling, warmth, pain, and potentially fever.[28-30] Alternative terms do exist in the literature to describe a wound that is critically colonised, including "sub-clinically infected", but at present there appears to be no consensus on how best to describe this stage in the wound infection continuum.[8,12,29] White and Cutting (2008) argued that critical

Key messages

- chronic wounds with biofilm, or "critically colonised wounds", are slow to heal and pose a significant burden to the patient and the healthcare system
- biofilms in chronic wounds are very often tolerant and resistant to antibiotics and antiseptics, leading to treatment failure
- a time-dependent "window of opportunity" is thought to exist after wound debridement during which biofilms are increasingly susceptible to treatment, in particular topical antiseptics
- the topical antiseptic PVP-I demonstrates a broad spectrum of antimicrobial activity, potent antibiofilm efficacy, no acquired bacterial resistance or cross-resistance, low cytotoxicity, good tolerability, and an ability to promote wound healing, making it a promising therapeutic option in wound care and biofilm management
- a new algorithm has been proposed for the management of chronic, non-healing wounds due to critical colonisation and/or biofilm, using PVP-I

colonisation is a cause of delayed healing and failure to acknowledge this important clinical step in the wound infection continuum could jeopardise the early diagnosis and treatment of a chronic wound.[29] Therefore, if a biofilm-infiltrated chronic wound can be treated before advancing beyond critical colonisation, this should not only improve the status of the wound but also help to avoid any significant personal and economic burden should the wound subsequently become infected.[29]

To date, therapeutic intervention to eradicate biofilms in chronic wounds has relied principally on the use of conventional antibiotics and antiseptics.[16] However, chronic wound biofilms can be highly tolerant and resistant to antibiotics and antiseptics.[21,31] A wide range of molecular mechanisms are thought to contribute to the recalcitrance of biofilms towards antimicrobial agents, which may subsequently lead to treatment failure.[31-35] Debridement also represents a viable treatment strategy against biofilms, but it is unable to completely remove all biofilm and so is not recommended for use alone.[21] Biofilms may reform quickly, even after repeated debridement, yet a time-dependent "window of opportunity" is thought to exist after debridement during which biofilms are more susceptible to treatment, in particular topical antiseptics.[21,31,36] By delaying biofilm reformation after debridement, topical antiseptics may reduce the risk of infection and subsequent need for antibiotics, helping to minimise the potential for antibiotic resistance to develop.[21] Indeed, recent consensus guidelines recommend topical antiseptics as first-line therapy in the treatment of stalled (chronic) wounds.[21]

Here, we review the antibiofilm efficacy, safety, and tolerability of topical antiseptics in chronic wound care and biofilm management, focusing specifically on povidone-iodine (PVP-I) in comparison with two commonly used antiseptics in wound care, polyhexamethylene biguanide/polyhexanide (PHMB), and silver. We also propose a new practical clinical guide or algorithm for the treatment of chronic, non-healing wounds due to critical colonisation or biofilm.

2 | METHODS

This narrative review results from a Focus Group meeting on "antiseptics in wound care and biofilm management" held in December 2019. The review is based primarily on the literature reviewed and recommended by the authors during the meeting. Additional English language publications of relevance were identified following literature searches conducted in PubMed in March 2020, using various combinations of the key terms: "antimicrobial", "biofilm", "critical colonisation", "chronic wound", "cytotoxicity", "non-healing wound", "polyhexamethylene biguanide", "polyhexanide", "polihexanide", "povidone iodine", "silver", "silver colloid", "silver compounds", "silver ion", "silver nanoparticles", "topical antiseptics", "wound dressing", and "wound healing". The key terms in all searches could be combined using Boolean operators such as "OR" or "AND". No date restrictions in the searches were employed. Only full-text articles identified from the searches that were considered directly relevant were included in this review, and most of these articles were open access. Reference lists of identified papers were also hand-searched to identify any further papers of interest. Reports and academic dissertations were not considered for inclusion in this review.

3 | RESULTS AND DISCUSSION

3.1 | Antimicrobial spectrum of activity

Differences observed in the antimicrobial spectrum of activity of PVP-I, PHMB, and silver may stem from the varying mechanisms of action of each antiseptic. An overview of the antimicrobial activity and mechanisms of action of PVP-I, PHMB, and silver is presented in Table 1.[37-70]

3.1.1 | Povidone-iodine

PVP-I is an iodophor or iodine-releasing agent, consisting of a complex of iodine and a neutral polymer base (polyvinylpyrrolidone), which acts as a reservoir of free active iodine.[37,44,67] PVP-I has a particularly broad antimicrobial spectrum of activity that includes Gram-positive and Gram-negative bacteria (including strains resistant to antiseptics and antibiotics), fungi, protozoa, viruses, bacterial spores, and amoeba.[37-43] Studies suggest that PVP-I exhibits a rapid onset of activity, with antimicrobial efficacy evident after a contact time of 1 minute, although there were certain study limitations to take into consideration.[71,72] Some studies have even demonstrated potent antimicrobial efficacy of PVP-I in as little as 15 seconds against certain enveloped viruses (eg, Ebola, Middle East Respiratory Syndrome coronavirus [MERS-CoV], and Severe Acute Respiratory Syndrome coronavirus [SARS-CoV]).[73-75] Of significance, a recent in vitro study has shown that PVP-I also provides rapid and potent activity against SARS-CoV-2, the virus responsible for the ongoing coronavirus disease 2019 (COVID-19) pandemic.[76] In the study, all topical and oral PVP-I products tested provided ≥99.99% virucidal activity against SARS-CoV-2 within 30 seconds of contact, suggesting that PVP-I could

TABLE 1 Antimicrobial activity and mechanisms of action for povidone-iodine, polyhexanide, and silver

Characteristic	Povidone-iodine	Polyhexanide	Silver
Spectrum of activity	Broad spectrum of activity against Gram-positive and Gram-negative bacteria, fungi, protozoa, viruses, bacterial spores, and amoeba[37-43]	Antimicrobial efficacy on Gram-negative bacteria, Gram-positive bacteria, and *Candida albicans*[51]	Bactericidal activity against Gram-negative and Gram-positive bacteria, fungi, and viruses[56-62]
Bacterial resistance	No reports of bacterial resistance[44,45]	No reports of bacterial resistance[51-53]	Gram-negative bacteria, including *E coli*, *P aeruginosa*, *K pneumoniae*, and *E cloacae*, may all express resistance[63-66]
Cross-resistance	No reports of cross-resistance to antibiotics or other antiseptics[40,46,47]	Prolonged exposure of MRSA to a low concentration of PHMB in vitro associated with reduced susceptibility, not only to PHMB, but also to daptomycin[54]	No cross-resistance reported[49]
Mechanism of action	Iodine oxidises fatty acids, amino acids, and nucleic acids, leading to destabilisation of cell membranes, deactivation of cytosolic enzymes, and disruption of internal metabolic pathways[48-50]	Disrupts cell membranes, increasing membrane permeability via interaction with acidic, negatively charged membrane phospholipids, and inhibits internal metabolic processes[54,55]	Ionic form reacts with the thiol groups in enzymes and proteins, adversely affecting enzyme function, cell replication, and energy generation in a non-specific manner[56,67-69] Silver ions also interfere with electron transport and/or membrane ion-exchange systems[69,70]

Abbreviations: MRSA, methicillin-resistant *Staphylococcus aureus*; PHMB, polyhexanide.

form a valuable part of future COVID-19 infection control strategies.[76] There have been no reports of bacterial resistance to iodine despite more than 150 years of use, possibly due to the multiple mechanisms of action exhibited by free iodine.[44,45,48,77] In addition, no evidence of cross-resistance to antibiotics or other antiseptics has been observed with PVP-I use in a wide range of Gram-positive and Gram-negative bacterial species.[40,46,47]

3.1.2 | Polyhexanide

PHMB is a biguanide, a strong base, which is highly positively charged at physiological pH.[51] Findings from in vitro studies have demonstrated efficacy of PHMB on Gram-negative bacteria, Gram-positive bacteria, and *Candida albicans*,[51] but it is without rapid onset of antimicrobial activity.[72] There have been no reports of bacteria acquiring resistance to PHMB,[51-53] possibly a consequence of its non-specific mechanisms of action.[55] Although recent reviews suggest no evidence of cross-resistance to antibiotics in Gram-positive and Gram-negative bacteria after low-level exposure to PHMB,[46,47] reduced susceptibility of methicillin-resistant *Staphylococcus aureus* (MRSA) to daptomycin and other cell wall-targeting antibiotics has been reported.[54]

3.1.3 | Silver-containing products

Silver-containing products used in wound care (eg, silver salts, colloidal silver, and more recently, silver nanoparticles) require the release of positively charged silver ions in order to exhibit antimicrobial activity.[68,78,79] The conversion process of inert metal to active ionised form is thought to be facilitated by interaction of the silver contained in wound dressings with aqueous media (eg, wound exudate).[80] The rate of onset of antimicrobial efficacy of certain higher silver release formulations has been demonstrated within 30 minutes of contact with clinically relevant bacteria.[81] Silver has demonstrated bactericidal activity against Gram-negative and Gram-positive bacteria, and may also target fungi and viruses.[56-62] Bacterial resistance to silver has been documented.[63-66] Evidence suggests silver resistance is encoded on plasmids.[82] Plasmid-encoded resistance to silver is of particular concern in the clinical setting as plasmid transfer between bacteria within polymicrobial-infected chronic wounds may confer silver resistance across multiple bacterial species.[83] Furthermore, plasmid-mediated metallic salt resistance has been shown to be associated with coresistance to antibiotics.[84] No cross-resistance to antibiotics has been reported to date.[49]

3.2 | Antibiofilm efficacy

3.2.1 | Povidone-iodine

The World Union of World Healing Societies 2016 Position Document recognises iodine as a suitable antimicrobial agent to manage biofilms,[18] and numerous studies have been conducted to investigate the antibiofilm efficacy of PVP-I. Sub-inhibitory concentrations of PVP-I (0.17%, 0.35%, and 0.7% w/v) inhibited biofilm development by *Staphylococcus epidermidis* and *S aureus*, two of the most prevalent bacterial species found within chronic wounds.[17,85] Using an in vitro model of chronic wound biofilms, Hill et al (2010) demonstrated that mixed *Pseudomonas* and *Staphylococcus* biofilms were disrupted by PVP-I 1% (w/v) solution; in contrast, these mixed biofilms were unaffected by treatment with ciprofloxacin and flucloxacillin.[86] Furthermore, in the same study, mature 7-day mixed biofilms were completely destroyed by PVP-I-containing dressing.[86] PVP-I at low doses (0.25% w/w) completely eradicated established biofilms of multi-drug resistant *S aureus*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa* and *C albicans* in vitro.[87] In a complex biofilm model, PVP-I 10% solution destroyed both early (90 minutes) and mature (48 hours) biofilms formed by *Candida auris*, an emerging multi-drug resistant yeast.[88,89] The antibiofilm efficacy of PVP-I is rapid in onset in vitro, with total eradication of mature 3-day biofilms of *S aureus* and *P aeruginosa* achieved after only 15 minutes' exposure.[90] Using a basally perfused biofilm model, PVP-I 10% w/v demonstrated greater effectiveness against a biofilm community consisting of *P aeruginosa*, *Streptococcus pyogenes*, MRSA, and *Bacteriodes fragilis* compared with PHMB 0.5% v/v and silver acetate 0.05% w/v.[91] In the study, PVP-I (57%) achieved the largest reduction in average bacterial count over time vs PHMB (44%) and silver acetate (27%).[91] In a further study, iodine-containing wound dressings demonstrated greater antimicrobial efficacy against mature biofilms of *P aeruginosa* and *S aureus* over a 24-hour period compared with silver-based dressings using an in vitro static diffusion model.[92]

3.2.2 | Polyhexanide

PHMB is the most used antiseptic to treat critically colonised and locally infected acute and chronic wounds.[93] Investigations into the antibiofilm efficacy of PHMB have demonstrated that wound irrigation by PHMB was effective against MRSA 3- and 6-day biofilms in a porcine wound model.[94] Application of a PHMB-containing biocellulose dressing to non-healing locally infected and/or critically colonised wounds provided good efficacy against existing biofilm in 10 (63%) adult patients, thereby facilitating wound healing.[95] In addition, comparable efficacy of PHMB to chlorhexidine (CHG) was demonstrated against *P aeruginosa* biofilm grown in routinely used microtitre plates and on silicone materials in vitro in artificial wound fluid.[96] However, although PHMB was equally as effective as saline solution in reducing the bacterial load in venous leg ulcers, neither treatment was able to eliminate biofilm from the wound tissue.[97] Furthermore, conflicting evidence exists regarding the activity of PHMB against *S aureus* biofilms.[98,99]

3.2.3 | Silver-containing products

Silver has been positioned among first-line options for the treatment of wound infections and is recommended for use in biofilm treatment.[12,18] Silver sulfadiazine 5 to 10 μg/mL eradicated mature *P aeruginosa* biofilm in vitro,[100] and colloidal silver 100 and 150 μL almost completely eliminated *S aureus* biofilm in vitro 24 hours after treatment.[101] Application of a silver-containing wound dressing to a 24-hour biofilm composed of either *P aeruginosa*, *Enterobacter cloacae*, *S aureus*, or a mixed bacterial community, resulted in total bacterial killing after 48 hours' exposure.[102] Antibiofilm efficacy of silver ions against *S epidermidis* is thought to be mediated by the binding of silver ions to proteins and polysaccharides within the EPS, leading to breakdown of the EPS and destabilisation of the overall biofilm structure.[103] Silver nanoparticles are a relatively new addition to the range of available antimicrobial treatment options.[104] Interaction of silver nanoparticles with an aqueous environment (eg, an exuding wound) promotes oxidation of the nanoparticles and subsequent release of antimicrobial silver ions.[104] A concentration-dependent effect on *P aeruginosa* biofilm development and architecture has been demonstrated, with complete inhibition of biofilm growth achieved with a high concentration (18 μg/mL) of silver nanoparticles.[105] Potent antibiofilm efficacy of silver nanoparticles has been shown against *C auris* by inhibition of biofilm formation and by the disruption and distortion of pre-formed biofilms.[106] Silver nanoparticles have demonstrated potent inhibition of biofilm formation by *Escherichia coli*, *P aeruginosa*, and *Serratia proteamaculans*.[107] A 7-day treatment in vitro study by Kostenko et al (2010) concluded that the efficacy of silver against biofilms formed by MRSA, *P aeruginosa*, and *E coli* was determined by the type of silver species and base materials used in the wound dressings.[108]

3.3 | Cytotoxicity and tolerability

A pre-requisite of any wound dressing is to shield the wound, offering not only a physical protective barrier but also a first line of defence against antimicrobial invasion of the wound. Intimate contact between the wound dressing and those cells that are key to the wound healing process is inevitable, so it is vital that a wound dressing demonstrates good cell compatibility.[109] Cytotoxic effects of a wound dressing would reduce the viability, proliferation, and migration of cells involved in the wound healing process, and so decrease the healing rate.[109] Studies have shown different levels of cytotoxicity among PVP-I, PHMB, and silver-containing products (Table 2).[110-129] Cytotoxicity data, in particular data derived from in vitro studies, must be interpreted with caution as any cytotoxic effects observed in cultured cell types can be magnified and may not be truly reflective of the in vivo or clinical setting.[49,130,131]

3.3.1 | Povidone-iodine

A cytotoxicity assay conducted in cultured murine fibroblasts showed that PVP-I had the lowest cytotoxicity compared with PHMB, silver nitrate, and silver sulfadiazine.[132] A further in vitro cytotoxicity test using murine fibroblasts demonstrated that PVP-I was less cytotoxic than a variety of antiseptics, including PHMB.[133] Furthermore, of the antiseptics tested, PVP-I was unique in provoking a revitalisation of fibroblasts, which may be pivotal to improved wound healing and better tissue tolerance with PVP-I.[133] In an in vitro study to investigate the cytotoxic effect of commonly used antiseptics on human fibroblasts and mesenchymal stromal cells, PVP-I was the only antiseptic to show remaining cell viability at the minimal bactericidal concentration (MBC; 1.32 g/L); in the same study, PHMB was 100% cytotoxic at the commercially available concentration of 0.04%, which was below the estimated MBC.[130] In vitro

TABLE 2 In vitro cytotoxicity of povidone-iodine, polyhexanide and silver-containing products against cell types instrumental to the wound healing process

Antiseptic	Fibroblasts	Keratinocytes	Endothelial cells
Povidone-iodine	Decrease in human fibroblast cell survival, cell migration, and cell viability[110,111]	Decrease in human keratinocyte cell viability[111]	Cytotoxic damage in bovine corneal endothelial cells[124]
Polyhexanide	Complete cell destruction of human skin fibroblasts[112] Time-dependent cytotoxicity in human dermal fibroblasts[113] High cytotoxicity in murine fibroblasts after up to 72 hours of incubation[114]	Concentration- and time-dependent cytotoxicity in human dermal keratinocytes[113] High cytotoxicity in human keratinocytes after up to 72 hours of incubation[114]	Large reduction in human endothelial cell number and viability[125]
Silver-containing products	Cytotoxic effects of silver-based dressings, silver nitrate, and silver ions in fibroblasts[115-117] Significant change in cell morphology and decrease in cell proliferation and collagen synthesis of human diabetic fibroblasts (silver-containing dressings)[118] Inhibition of human dermal fibroblast proliferation with silver-dependent cell loss (silver nitrate)[119] Time- and concentration-dependent cytotoxicity in human fibroblasts (silver nitrate)[120] Dose- and time-dependent cytotoxicity in human periodontal fibroblasts (silver nanoparticles)[121]	Cytotoxic effects of silver-based dressings, silver nitrate, and silver ions in keratinocytes[115-117] Inhibition of human keratinocyte growth (nanocrystalline silver dressing)[122] Decrease in human epidermal keratinocyte viability, metabolism, and proliferatory and migratory potential (silver nanoparticles)[123]	Formation of reactive oxygen species and induction of apoptosis in human umbilical vein endothelial cells (silver nanoparticles)[126,127] Dose-dependent inhibition of the proliferation of rat vascular endothelial cells (silver nanoparticles)[128] Increase in human umbilical vein endothelial cell membrane permeability (silver nanoparticles)[127,129]

evidence does exist to suggest that PVP-I may also be cytotoxic towards certain cell types involved in the wound healing process, including fibroblasts, keratinocytes, and endothelial cells.[110,111,124] However, Bigliardi et al (2017) commented that PVP-I is well tolerated in human studies when used in appropriate concentrations, and that pronounced cytotoxicity with PVP-I has only been observed in certain in vitro studies.[134] Indeed, the densities of dendrocytes and microvessels in chronic leg ulcers were higher following 6 weeks of PVP-I treatment compared with silver sulfadiazine and CHG, with no evidence of dendrocytoclasis, which may be considered a sign of in vivo cytotoxicity.[135] In contrast, silver sulfadiazine and CHG promoted changes in the superficial microvasculature and induced dendrocytoclasis.[135] Overall, PVP-I has a good tolerability profile.[49] The prevalence of allergic contact dermatitis caused by PVP-I has been estimated to be approximately 0.4%, with reports of anaphylaxis exceptionally rare.[136]

3.3.2 | Polyhexanide

There are reports to suggest that PHMB is cytotoxic towards cells that are crucial to the wound healing process. A range of concentrations of PHMB (0.005%-1.0% v/v) demonstrated high cytotoxicity against cultured human keratinocytes and murine fibroblasts after 24 and 72 hours of incubation in vitro.[114] In an in vitro cytotoxicity test using cultured human skin fibroblasts, PHMB was extremely cytotoxic and appeared to induce complete cell destruction in the majority of cells.[112] When used in vitro at or below concentrations commonly employed in human wound care, PHMB (0.01%, 0.04%, and 0.1%) demonstrated both time- and concentration-dependent cytotoxicity in cultured human keratinocytes and osteoblasts, but only time-dependent cytotoxicity in cultured fibroblasts.[113] Furthermore, exposure of cultured human endothelial cells to PHMB (0.0006%-0.01%) resulted in a large reduction in cell number and viability.[125] The overall tolerability profile of PHMB is good, with report of contact dermatitis found to be rare.[136] However, a recent case report identified PHMB as an emerging allergen, which may have induced an anaphylactic reaction.[137]

3.3.3 | Silver-containing products

Studies have indicated cytotoxic effects of silver-based dressings and silver nitrate in both cultured fibroblasts and keratinocytes,[115] with silver-based dressings also shown to cause a significant delay in re-epithelialisation.[116] In addition, silver-based dressings have been shown to significantly alter the cell morphology and decrease cell proliferation and collagen synthesis of cultured human diabetic fibroblasts in vitro compared with silver-free dressings, suggesting the use of such dressings should be closely monitored when treating diabetic wounds.[118] Although the cytotoxic effects of silver nanoparticles are thought to be dependent on a number of factors, including nanoparticle size, shape, concentration, and aggregation, it remains to be determined if the cytotoxicity is due to inherent properties of the nanoparticles themselves or due to the release of ionic silver following oxidation.[138-140] Indeed, ionic silver has been found to be significantly more cytotoxic in vitro in human dermal fibroblasts and epidermal keratinocytes compared with silver nanoparticles.[117] Additional in vitro studies provide further evidence of the cytotoxicity of silver-based products on fibroblasts, keratinocytes, and endothelial cells.[119-123,126-129] Nevertheless, silver has a good tolerability profile, and although argyria can result from the long-term use of silver-based products, any discolouration of the skin is not thought to result in pathological tissue damage or to be in any way a danger to life.[141]

3.4 | Wound management

In wound management, it is important to not only consider the antimicrobial efficacy and potential cytotoxicity of antiseptics, but also to be aware of the way in which antiseptics may impact the complex cellular and extracellular mechanisms involved in the wound healing process.[142] One of the many properties an ideal antiseptic should possess is its ability to facilitate wound healing.[49]

3.4.1 | Povidone-iodine

PVP-I enhanced wound healing via increased expression of transforming growth factor-β (TGF-β), neovascularisation, and re-epithelialisation in a rat acute skin wound model.[143] In vitro evidence suggests that PVP-I may facilitate wound healing by exerting an anti-inflammatory effect, scavenging superoxide anions, and inhibiting the production of reactive oxygen species by human polymorphonuclear neutrophils.[144] Indeed, treatment of venous leg ulcers with PVP-I in combination with hydrocolloid dressing reduced bacteria-related inflammation, vasculitis, and phagocytic infiltration of the ulcers compared with hydrocolloid dressing alone, resulting in an improved ulcer healing rate.[145] In a further study, the healing rate of chronic leg ulcers was significantly increased by PVP-I vs controls, reducing the

time to healing by 2 to 9 weeks.[135] Recently, a phase IV prospective study conducted in 106 adult patients showed that PVP-I foam dressing achieved a shorter epithelialisation time compared with hydrocellular foam dressing and conventional petrolatum gauze when used as a split-thickness skin graft donor site dressing.[146] Further evidence supports the role of PVP-I in wound healing when investigated within in vivo human studies.[147,148] Compared with silver-based foam dressings or control gauze, PVP-I 3% foam dressing was the most effective dressing in wound healing by promoting neovascularisation, re-epithelialisation, and collagen deposition in an in vivo rat wound model.[149] Similarly, PVP-I 10% solution promoted rapid neovascularisation more effectively than silver nitrate solution in an in vivo mouse wound model.[150]

3.4.2 | Polyhexanide

Evidence to date suggests that PHMB may also be beneficial for wound healing. In a recent systematic review, it was concluded that PHMB may promote the healing of chronic wounds, reduce the bacterial load, eradicate MRSA, and lessen wound-related pain.[151] A preclinical study using a mouse wound model demonstrated that PHMB had more beneficial effects on the microcirculation, angiogenesis, and epithelialisation compared with chitosan.[152] In a further study, PHMB had a more positive effect upon the blood flow of intact human skin in vivo than octenidine, suggesting value in the treatment of critically perfused wounds, such as burns.[142] Comparison of a PHMB-containing dressing with a silver-based dressing in patients with critically colonised and locally infected wounds demonstrated that the PHMB-containing dressing was significantly faster and more effective at removing the critical bacterial load over a 28-day period.[153] In addition, PHMB has been shown to protect keratinocytes from bacterial damage by *S aureus* and re-establish normal cell proliferation in vitro in a dose-dependent manner.[154] Nevertheless, a recent in vitro study has suggested that PHMB may exert pro-inflammatory effects, including increased cytokine secretion and nuclear factor kappa B activation,[155] both of which would hinder the wound healing process.

3.4.3 | Silver-containing products

A Cochrane meta-analysis concluded that there is insufficient evidence to confirm whether silver-containing products can promote wound healing.[156] Furthermore, the heterogeneous nature of the evidence regarding the effectiveness of silver-based treatments in wound care is thought to have hindered the development of treatment guidelines.[157,158] As such, authors of a recent qualitative literature analysis proposed that silver-containing wound dressings should be chosen with care if the wound healing process is not to be impeded.[157] Nevertheless, there is evidence to suggest that silver can have beneficial effects on the healing of chronic wounds, including those wounds showing signs of critical colonisation.[159,160] For example, Duan et al demonstrated that a sub-cytotoxic concentration of silver ions may promote the proliferation of human skin keratinocytes in vitro.[161] Additionally, silver nanoparticles decreased the generation of pro-inflammatory cytokines by human keratinocytes and fibroblasts in an in vitro wound healing model.[162]

3.5 | Algorithm for the treatment of chronic, non-healing wounds due to critical colonisation or biofilm

Of the three antiseptics discussed in this review, PVP-I has particular characteristics that are ideal for the treatment of chronic, non-healing wounds due to critical colonisation and/or biofilm, namely its potent antibiofilm efficacy, broad spectrum of antimicrobial activity, wound healing properties, and rapidity of action. Therefore, we have proposed a new practical clinical guide or algorithm to remove biofilm and manage critically colonised wounds, using PVP-I (Figure 1).

When biofilm presence within a chronic wound is strongly suspected, clinicians should adopt an early intervention plan to remove the biofilm as soon as possible and reduce the risk of infection.[21,23] Wound bed preparation is vital if successful wound healing is to occur, as described in the TIMERS (Tissue, Inflammation/infection, Moisture imbalance, Epithelial edge advancement, Repair/regeneration, and Social factors) framework to guide wound care (Figure 2).[23,163] According to the new algorithm proposed here, intensive mechanical washing or cleansing of the wound with either soap or PVP-I scrub should help to prepare the wound bed by removing debris and biofilm from the wound. This should preferably be performed without causing any additional trauma to the wound.[26] Ideally, cleansing of the wound should be performed with each change in dressing.[12] This is particularly the case if the likelihood of biofilm is high, but the unique characteristics of each particular wound bed should determine the frequency of dressing changes. Following wound cleansing, debridement of the wound can help to disrupt any remaining biofilm, remove necrotic tissue, and stimulate wound healing.[12,30,163] Debridement may be achieved using a number of different methods,

FIGURE 1 A proposed new algorithm for the treatment of chronic, non-healing wounds due to critical colonisation and/or biofilm. *Secondary dressing is to be used to keep the primary dressing securely in place

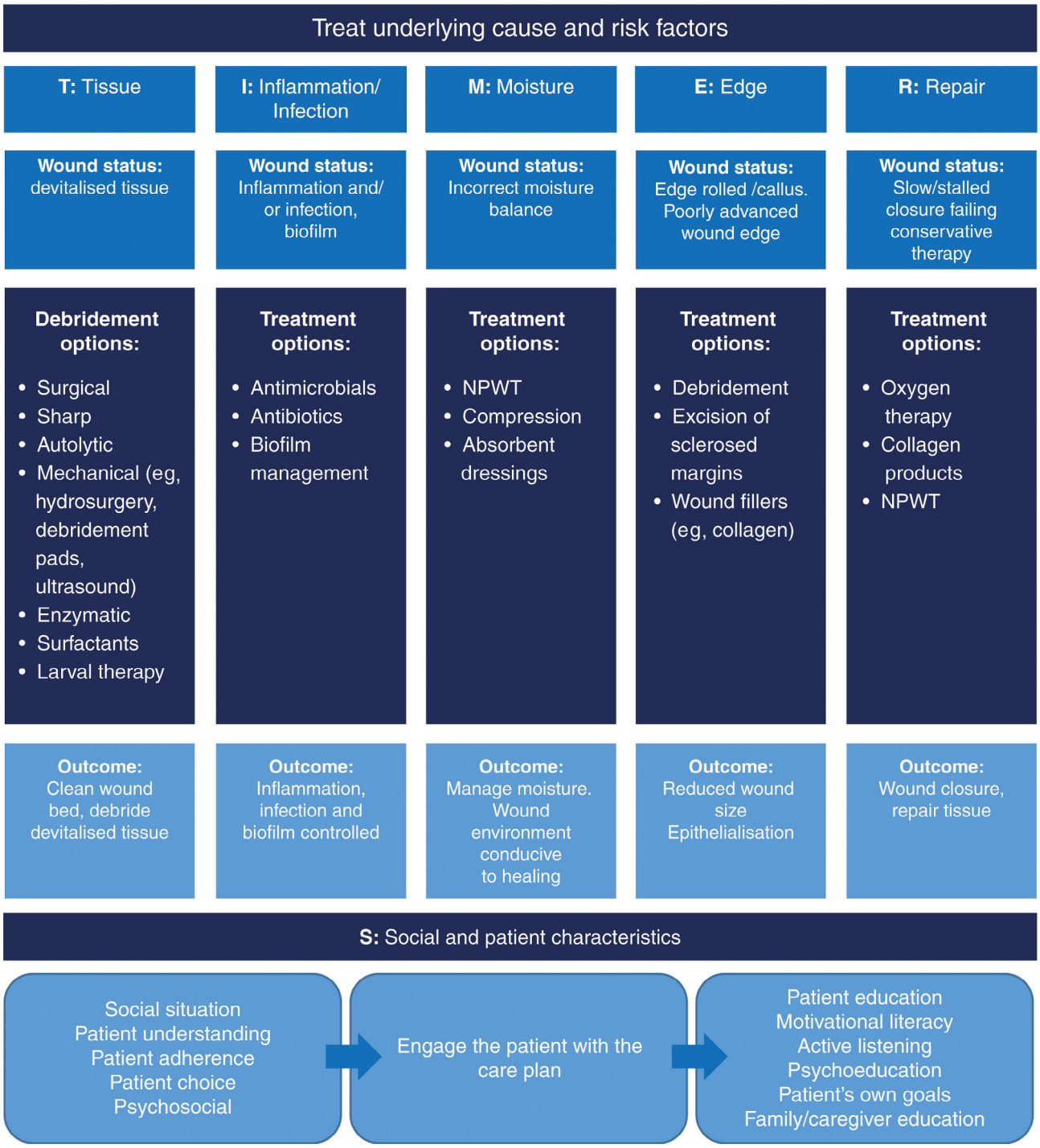

FIGURE 2 TIMERS framework for the management of chronic, non-healing wounds. (Data reproduced with permission of MA Healthcare Limited, from Atkin et al, 2019; permission conveyed through Copyrigh Clearance Center, Inc.).[23] NPWT, negative pressure wound therapy

including surgical, mechanical, and chemical techniques.[12] The wound can then be disinfected using gauze impregnated with PVP-I dermic solution. Given the rapid onset of action of PVP-I, employing a contact time of at least 1 minute may be sufficient to eradicate the majority of any microbes remaining in the wound.[71,72]

Biofilm is not completely removed by debridement and it may quickly regrow within 24 hours, so debridement alone is not an appropriate treatment strategy.[21,36] Regrowth of biofilm must be controlled according to the status of the wound, in particular the amount of exudate the wound is producing. A balanced, moist wound

environment is seen as critical for wound healing.[23] Not enough exudate or excessive production of exudate will hinder the wound healing process.[23] Dry wounds are devoid of exudate, which hinders the activity of tissue-repairing cells (Figure 3).[163] With low and moderate exuding wounds, the wound bed and surrounding skin become increasingly wet. The excessive amount of exudate produced by a highly exuding wound may result in maceration of the surrounding skin.[30] Therefore, it is important to manage moisture levels by selecting the correct dressing.[23] Numerous types of dressing are available to both protect the wound and promote healing (Table 3).[1,164] Choosing the most appropriate option from the extensive range of dressings available can be a difficult treatment decision, but it should ultimately be tailored to the characteristics of each wound and to the patient.[23,30] Dressings that manage the exudate and encourage a balanced wound environment are crucial for improved patient outcomes.[165] The new algorithm proposes that low to moderate exuding wounds can be treated with PVP-I gel and PVP-I tulle and covered with a secondary dressing. Highly exuding wounds may be treated with PVP-I gel applied beneath an absorbent dressing. Until signs of improvement in the wound bed surface are evident, dressings should be changed daily and regularly checked for discolouration, as any change in colour can indicate reapplication of PVP-I is needed in order to maintain its clinical efficacy.[166] Regular monitoring of the healing status of the wound is required according to specific criteria, including assessment of the size and depth of the wound, and the amount and type of exudate.[30] If there is an improvement in wound healing,

FIGURE 3 Different types of exuding wound. Images were kindly provided by the authors P.J.A. and S.M., with permission

TABLE 3 Different types of wound dressing currently available for wound management and their potential clinical uses

Level of wound exudate	Type of dressing	Properties	Clinical use
Low	Hydrogels	Rehydrates dry wounds, easily removed/changed, may cause overhydration, removes necrotic tissue	Dry wounds
	Films	Occlusive, retains moisture, only for non-exudative wounds	Superficial wounds with very limited exudate
	Gauze	Inexpensive, drying, may cause further injury upon changing	Clean and dry wounds with low exudate levels
	Hydrocolloids	Long-lasting, keep moist environment, not for use on wounds with high exudate levels due to impermeable nature, occlusive, not for infected wounds	Low-to-moderate drainage wounds
	Foams	Moderately absorbent, insulating, for use on moderately exuding wounds, minimal trauma during dressing changes	Low-to-moderate drainage wounds
	Gelling fibres	Highly absorbent	Moderate-to-highly exuding wounds
	Polyacrylate polymers	Highly absorbent	Highly exuding wounds
High	Alginates	Highly absorbent, haemostatic	Infected and non-infected wounds with large amount of exudate

Note: Data obtained from Han and Ceilley, and Shi et al.[1,164]

treatment may revert to the standard of care (Figure 2).[23] Reassessment of the wound should be made on a weekly basis and, if necessary, the procedure outlined in the algorithm can be restarted.

4 | CONCLUSIONS

Recent evidence suggests that the majority of chronic wounds have biofilms which can hinder wound healing and result in ineffective treatment, burdening both the patient and the healthcare system. PVP-I demonstrates potent efficacy against biofilms formed by a variety of microbes found to be prevalent within chronic wounds, including *S aureus*, *S epidermidis*, and *P aeruginosa*. Given how diverse the microbial community can be within chronic wounds, the broader spectrum of antimicrobial activity of PVP-I should be advantageous vs the more limited spectrum of antimicrobial activity shown by PHMB and silver-containing products. PVP-I also fulfils all of the other requirements of an ideal antiseptic for chronic wound care, including a lack of acquired bacterial resistance or cross-resistance, wound healing properties, low cytotoxicity, and good tolerability. Collectively, these characteristics of PVP-I suggest that it represents a highly viable therapeutic option in wound care and biofilm management, with the potential to be particularly effective during the critically colonised, biofilm-infiltrated stage of the wound infection continuum. The proposed new algorithm utilising PVP-I should help to guide clinicians in the treatment of patients with chronic, non-healing wounds, which prove particularly unresponsive to treatment.

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CONFLICT OF INTEREST

P.J.A. has served as a consultant for Viatris. R.T.B. has served as a consultant for Viatris. B.M.B. has served as a consultant for Viatris. L.G.G. has served as a consultant for Viatris. S.M. has no conflicts of interest. S.J.M. has received honoraria as a speaker and has been a member of advisory boards for Viatris.

AUTHOR CONTRIBUTIONS

All authors contributed to developing the algorithm, analysis and interpretation of information presented, drafting and revising the article, gave final approval of

the version to be published, and agree to be held accountable for all aspects of the work.

DATA AVAILABILITY STATEMENT

Data sharing is not applicable to this article

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